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APPLICATION OF UMBILICAL CORD BLOOD TRANSFUSION IN POST-COVID-19 ANAEMIC PATIENTS

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ABSTRACT

The practice of discarding Umbilical Cord Blood (UCB) is very common in 3rd world countries but still a major issue in developing countries like India. UCB transfusion is done in post-Covid patients to treat anaemia, and there are various ethical issues which have to be mitigated. In this study we compared the clinical symptoms of people who received UCB for the treatment of anaemia and the people who received other standard of care for anaemia after post-Covid 19 infections. All patients were diagnosed as Covid-19 by RT-PCR test results. Symptomatic patients with complete blood count report suggestive of anaemia were included in our study. UCB was collected aseptically from surgical operation theatre. Available group matched patients were selected for transfusion after taken proper consent. All patients were followed up for consecutive four months. Results showed that the patients with cord blood transfusion recovered earlier than the patients managed by standard of care.

Keywords: Anaemia, covid-19, umbilical cord blood transfusion

INTRODUCTION

The novel Corona virus disease 2019 (Covid-19) is a communicable disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Having incubation period of 1-14 days, the disease commonly shows the symptoms of fever, cough, fatigue, breathing difficulties, anaemia, cytopenia and loss of taste and smell (Suvvari *et al.,* 2019). Majority of Covid-19 patients are asymptomatic or have mild symptoms and eventually recover. Some Covid-19 patients experience symptoms long after their Covid-19 polymerase chain reaction test (RT-PCR) turn negative; this is commonly referred to as "post-Covid-19 syndrome" or "long Covid." As per the National Institute for Health and Care Excellence (NICE), post-Covid-19 syndrome is defined as "the signs and symptoms that develop during or after an infection consistent with Covid-19, continuing for more than 3 months, and not explained by an alternative diagnosis" (Suvvari *et al.,* 2021). Freshly collected human umbilical cord blood (HUCB) contains on an average of 40-60 mL blood at term. HUCB is rich in foetal haemoglobin (HbF) that has the potential to carry 60% more oxygen than adult haemoglobin (Hb). HUCB also has higher platelet count of 750000 against 250000 μL^{-1} of adult blood and WBC count of 24,000 against 6500-10,500 cells μL^{-1} in adult blood (Bhattacharya, 2006). UCB has been found to contain an array of anti-inflammatory cytokines like IL-10, IL-4, etc. (McNalley *et al.,* 2008). It is hypothesized that transfusion of freshly collected cord blood, by virtue of its unique characteristics and composition, can improve anaemia and other post-Covid related conditions.

Halbrecht in 1939 first reported his unsuccessful cord blood transfusion attempt ((Halbrecht *et al.* 1939). However, the absence of anticoagulant like acid citrate dextrose resulted in clot formation

and the concept of screening blood for different transfusion-related transmitted infectious diseases was not prevalent at that time. In 1999, researchers at Calcutta (India) showed the safety and efficacy of cord blood transfusion in a large group of patients (Bhattacharya *et al.*, 2006). Prof Elaine Gluckman on her visit to Calcutta inspected and reported the successful transfusion of freshly collected blood group matched, HLA randomized and thoroughly screened cord blood in anaemic patients suffering from malaria, tuberculosis, cancer, beta thalassemia, leprosy, HIV, diabetes, and arthritis (Bhattacharya *et al.*, 2005a; Bhattacharya, 2006, Bhattacharya *et al*., 2006). Another group from UK in 2003 similarly reported the safe use of cord blood transfusion in many sub-Saharan children who did not had access to adult blood transfusion and suffered from severe anaemia in the background of malaria (Bhattacharya *et al*., 2001). UCB transfusion induces rises in haemoglobin by nearly 21% from base line haemoglobin level (Bhattacharya *et al.*, 2005b).

In most cases, 99.9% cord blood is discarded and only 0.01% nucleated cells are used for transplantation studies or cord blood banking (Killimarti *et al.*, 1976). The placental vessels at term contain about 120 mL of cord blood and predominantly consist of foetal haemoglobin (HbF), whereas adult haemoglobin (HbA) accounts for only 15-40% and HbA2 only in trace amounts at birth (Osaki *et al*., 1982; Patel *et al*., 2022). HbF has greater oxygen binding affinity than HbA with a shift in the oxygen dissociation curve. This greater affinity for oxygen is explained by the lower levels of 2, 3-diphosphoglycerate in HbF unlike in HbA, where the oxygen affinity is lower due to higher concentration of 2, 3-diphosphoglycerate (Delivoria Padopoulos *et al.*, 1971). This higher oxygen binding capacity of HbF can be utilized in ischemic and a wide variety of diseases including anaemia.

HbA consists of two each of α and β polypeptide chains, each bound to a heme group, capable of binding with one molecule of O_2 (1 g haemoglobin binds with 1.39 mL O_2). Therefore, 14% adult haemoglobin carry, on an average, 19.46 mL O_2. On the other hand, cord blood at term carries, on an average, 16.8 g% haemoglobin, of which 20% belongs to HbA type (3.36 g), and 80% to HbF type (13.44 g) (Hassall *et al*., 2003). The concentration of HbF may increase further depending on the foetal stress, maturity, and several other feto-maternal factors. HbF has the potential to carry 50–60% more O_2 than HbA (Delivoria Padopoulos *et al.*, 1971). HbF per g may carry up to 2.08 mL O_2. If one calculates the O_2-carrying capacity of 100 mL of cord blood theoretically, taking into consideration that it contains 80% HbF and 20% HbA, it would be around 32.62 mL. This means that cord blood has 67.62% more O_2-carrying capacity than adult blood, which has around 19.46 O_2 100 mL^{-1}. The present study was aimed to assess the safety of umbilical cord blood transfusion and assess whether there are any immunologically related adverse events; any changes in the baseline level of haemoglobin and post COVID-19 related complications; after cord blood transfusion in an individual.

MATERIALS AND METHODS

Study subject

A total of twelve COVID-19 RT-PCR positive anemic patients (aged 18 to 70 years) volunteered for this study. Amongst them, six COVID-19 symptomatic anemic patients received human umbilical cord blood transfusion while six RT-PCR positive COVID-19 anemic patients served as control group and were given hematinic for one month for correction of anemia. The study was conducted in RMTS, Carmichael Hospital, School of Tropical Medicine (STM) and Hospital, Kolkata, West Bengal (India) in 2020-2021. The patients admitted in the Department of RMTS, STM during 2020-2021 were the subjects of study. The study was conducted after obtaining proper ethical clearance from the College vide IEC ref No: CREC-STM/2020-AS-27 dated 18 August, 2020.

Study design

Both male and female post-COVID volunteer patients aged 18-70 years and having haemoglobin levels $\leq$ 10 g dL^{-1} were selected for the present study. The patients suffering from COVID-19 pneumonia and severe anaemic patients requiring urgent blood transfusion, those not adhering to the

study protocol and those missing the follow up visits as per the decision made by expert clinical team were excluded from this study. Anaemic patients due to unknown causes, haemoglobinpathies and systemic disease suffering patients and those having the presence of other chronic ailments e,g CKD, COPD, hypertension, malignancy were also excluded from the study. The control group comprised of 6 post covid-19 patients with anaemia who had been treated with haematinics and other standard therapy as decided by the clinical expert team in the department. The experimental group consisted of 6 post covid-19 patients with anaemia who had been transfused with freshly collected human umbilical cord blood (UCB). The study was conducted up-to 4 months from the day 0 or baseline study day. All the participants were explained about the study protocols and the risk factors involved and a written informed consent was obtained from each subject for their volunteer participation in the study.

Parameters studied

The temperature of patients on each visit was regularly monitored by thermal gun (Haier R1B1) during clinical examination. Pulse oximeter (RoHS CE, XECH, Japan) was used for monitoring the oxygen saturation, pulse rate and respiration on each visit. O_2 saturation was estimated in blood via infrared technique by placing it on the index finger of patient in restive state (Fig. 1). Blood samples were drawn on each visit from antecubital vein in a dry K3 EDTA vacutainer for complete blood count by cell counter (Sysmex, Erba, TransAsia) and clotted blood samples were collected for serum ferritin estimation by ELISA. Symptoms of fever, cough, taste, smell, oxygen saturation, sore throat, skin manifestation, digestive systems, psychological symptoms, neurological symptoms, headache and chronic pain were studied and analysed by using severity score index (SSI parameters) [Zhang *et al.* 2020]. The severity index score of 0-4 was adopted for fever with 0 = normal (<37.2°C); 1= mild/ low grade fever (>37.3-38.0°C); 2 = moderate fever (>38.1-39.0°C); 3 = high grade fever (>39.1-41.0°C); and 4 = hyperpyrexia (40°C). For cough 0-3 score was followed with 0 = no cough, 1 = mild cough (once or twice), 2 = moderate (> 4 cough sustained for 2 sec.) and 3 = severe cough (> 4 coughs sustained for 4 sec). Taste and smell were categorized into no taste/ smell loss score = 0; and loss of taste/smell score = 1. For oxygen saturation, O_2 level ≥94% was graded = 0, O_2 level between 92-94% = 1; and O_2 level < 92% = 2. The severity index for sore throat adopted was: 0 = no sore throat, 1 = mild sore throat, 2 = moderate sore throat; and 3 = severe sore throat. Severity index for skin manifestation followed was: 0 = no rash, 1 = erythematous rash, 2 = vesicular rash; and 3 = urticarial rash. The severity index for digestive system followed was: 0 = no symptoms, 1 = nausea, vomiting or any one, and 2 = diarrhea, abdominal pain or any one. The severity index for psychological symptoms adopted was: 0 = no symptoms, 1 = anxiety, and 2 = depression, and 3 = post-traumatic stress disorders. For neurological symptoms the severity index used was: 0 = mentally alert, no myalgia or fatigue, 1 = myalgia, fatigue or any one, and 2 = altered mental status or delirium. The severity index for headache followed was: 0 = no headache, 1 = migraine, tension headache or any one, and 2 = cough headache at fronto-partial region. For chronic pain the severity index used was: 0 = no pain, 1 = post-Covid headache, joint pain or any one, and 2 = post-Covid testicular pain diarrhea, and 3 = chest pain.

Collection of cord blood

The collection of UCB was done immediately at baby's birth (followed the SOP of cord blood bank; license No-DL-2MB/SLA/CLAA/WB). Prior to the collection, phlebotomist confirmed the identity of donor. After delivery of baby and just after transection of cord between clams the phlebotomist cleaned the cord site with 70% IPA or 70% ethanol following the standard sterile blood collection protocol. For taking out the UCB collection bag from kit box, the expiry date of bag, content and leakage were checked. For blood collection the needle was inserted into the umbilical cord through already cleaned site and collection bag was kept a bit lower than the puncture level so that blood flows freely into it in favour of gravity. The milking of cord was done in cases of low amount of cord blood. Gentle rocking was done to prevent coagulation and proper mixing of anticoagulant. As soon as blood stopped tickling, the clamp was placed above the puncture site. At least 3 times knots were placed subsequently. The collection bags were wrapped with clean wipe placed in aluminium foil bag in between the ice pack along with the consent form and duly filled in collection booklet, wrapped in

Fig. 1: Visual inspection of empty cord blood bag (top left side); collection of human umbilical cord blood by Dr. Mainuddin Naskar, senior Obstetrician with team at operation theatre of Vidyasagar State General Hospital, Behala, Kolkata (top right side); post collection bag for collection of sample for quality control (bottom lefts side), and estimation of volume collected (bottom right side).

plastic bag on one side of the bag inside the collection kit box (Fig. 1).

Application of cord blood in anaemic patients

The collected bags were stored at 4°C. A sample from the collected blood was drawn for blood groups Rh and ABO, and matching, cross reactivity, HIV (1 and 2), hepatitis B and C, VDRL, malaria detection done as per the standard blood transfusion protocol [gazette notification dated 2nd February 20217 pertaining to the blood bank]. In case of any contamination or confusion, the concerned culture was put aside for the identification of pathogen, if any, through appropriate protocol [screening donated blood transfusion transmitted infections 2009]. The blood bags that matched the sample were marked unfit for transfusion. Post-Covid-healed patients with haemoglobin count < 8 g dL^{-1} were confirmed through hemogram tests; and admitted in the Department and transfused with cord blood or adult blood on day care basis. The patients were observed carefully for initial 1 h or so for, any transfusion-related reactions. Thereafter, if no problem persisted, the transfusion rate was enhanced until completion. The follow up studies were conducted by a group of medical officers and post-graduate students in the department. Follow up studies included complete hemogram, serum ferritin, liver and renal function test, and oxygen saturation.

Statistical analysis

Prior to clinical symptoms, the variables were assessed and analyzed for comparison between groups, using standard software excel SPSS, IBM 20 and DataTab for descriptive statistics and frequencies.

RESULTS AND DISCUSSION

Analysis of disease symptom score

A total of 12 Covid-19 RT-PCR positive anemic patients aged between 18 to 70 years volunteered for this study. Amongst them, 6 Covid-19 symptomatic anemic patients received the human umbilical

cord blood transfusion as per availability. The other 6 RT-PCR positive Covid-19 anemic patients served as control and were given hematinic for one month for correction of anemia. Each patient from each group was thoroughly examined for 120 days for above parameters as well as the data from their blood reports and clinical symptoms were collected.

Analysis of common Covid-19 symptoms

Wilcoxon signed rank test (non-parametric) was used to compare the variables fever, cough, loss of taste, loss of smell/taste. The data was not normally distributed. There was significant median difference between the follow-up and initial study groups with respect to fever, cough, loss of taste, loss of smell, etc. at 0.05 level of significance with p values of 0.023, 0.023, 0.014 and 0.014, respectively; which was less than 0.05. There was a statistically significant median decrease in fever, cough, loss of taste and smell when patients accepted the treatment as compared to the initial stage of treatment (Table 1). The median decreases by 2.0, 2.0, 1.0, and 1.0 in the above respective disease symptoms with initial versus follow up after giving umbilical cord blood treatment (Fig. 1).

Table 1: Comparative statistical analysis of common Covid-19 in control and experimental group

	Control group (n = 6)			Experimental group (n = 6)			
Symptoms	Initial mean	Follow up mean	Statistical significand	Initial mean	Follow up mean	Significance	Statistics
Fever	2.33	0.08	0.026	2.33	0.00	0.023	<0.05
Cough	2.00	1.25	0.041	2.33	0.00	0.023	<0.05
Taste	0.67	0.17	0.083	1.00	0.00	0.014	<0.05
Smell	0.83	0.17	0.046	1.00	0.00	0.023	<0.05

Analysis of other Covid -19 related atypical symptoms

Wilcoxon signed rank test (non-parametric) was used to compare the variables like skin lesion, digestive symptoms, neuro-psychological manifestation, headache with chronic pain and sore throat. Perusal of Table 2 revealed that the data was not normally distributed. There was significant median difference between follow-up and initial study groups with respect to the skin lesion, digestive symptoms, psychological manifestation, headache, chronic pain, and sore throat at 0.05 level of significance with p values of 0.014, 0.026 and 0.014, 0.020, 0.024 and 0.023, respectively, which was less than 0.05. There was a significant median decrease in skin lesion, digestive symptoms, psychological manifestations, headache, chronic pain, and sore throat when the patients accepted the treatment as compared to the initial stage of treatment (Table 2). The median decreases by 1.0, 1.5, 1.0, 1.0, 1.0, and 2.0 in above respective variable symptoms with initial versus the follow up after giving umbilical cord blood treatment. There was no significant difference between follow up and initial study groups to neurological symptoms at 5% level of significance, the p value was 1.0 which was greater than 0.05.

Analysis of haemoglobin (Hb) level in Covid-19 patients in control vs experimental group

There was significant median difference between Hb follow up and Hb initial study groups at 5%

Table 2: Comparative statistical analysis of atypical Covid-19 with control and experimental group

	Control group (n = 6)			Experimental group (n = 6)			
Symptoms	Initial mean	Follow up mean	Statistical significance	Initial mean	Follow up mean	Statistical significance	Statistics
Skin lesion	0.33	0.42	0.065	1.00	0.00	0.014	< 0.05
Digestive symptoms	2.00	0.92	0.0.39	2.00	0.50	0.026	< 0.05
Psychological symptoms	0.67	0.33	0.157	1.00	0.00	0.014	< 0.05
Neurological symptoms	0.00	0.33	0.157	0.00	0.00	1.000	≥ 0.05
Headache	1.83	1.00	0.059	1.17	0.00	0.020	< 0.05
Chronic pain	1.50	1.00	0.102	2.00	0.75	0.024	≥ 0.05
Sore throat	0.33	0.00	0.317	2.00	0.17	0.023	≥ 0.05

level of significance as the p-value of 0.046 was less than 0.05. There was significant median increase in Hb (8.89 g dL^{-1}.) when patients accepted the treatment as compared to the initial stage of treatment (8.20 g dL^{-1}). The median increase in Hb was 0.92 g dL^{-1} with initial versus follow up after giving umbilical cord blood treatment (Table 3).

Table 3: Comparative statistical analysis of hemoglobin (Hb) in COVID-19 with control and experimental groups

	Control group (n = 6)			Experimental group (n = 6)			
Symptoms	Initial mean	Follow up mean	Statistical significance	Initial mean	Follow up mean	Statistical significance	Statistics
Hemoglobin	8.92	9.61	0.0249	8.12	8.88	0.046	<0.05

Conclusions: As in third world country like in India Umbilical Cord Blood is often discarded and seldom stored for the purpose of future use. Our study shows collection of Umbilical Cord Blood and storage due to unique composition of Umbilical Cord Blood it could be safely used for the purpose of transfusion amongst Post COVID patients. Our study had indicated that there is better outcome in clinical symptoms in the intervention arm compared to control arm. The difference in median increase in Sr. Hb in control arm compared (0.94 g dL^{-1}) to intervention arm might have happened because of a smaller number of volunteers in intervention Arm. The volunteer was randomly assigned into two arms following the inclusion criteria and exclusion criteria of this study. The outcome of our study clearly demands further investigation into this topic. Moreover, transfusion of UCB can potentially mitigate the issue of scarcity blood donors that we had faced during the time of pandemic.

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